Are no. B? seq. list? placed before us.

## REMARKS

**Double Patenting**

Under the doctrine of obviousness-type double patenting, the examiner has provisionally rejected claims 1-43 and 52 over claims 1-32 and 35-47 of U.S. application serial No. 09/714438. While applicants do not acquiesce to the stated rationale for this rejection, they are amenable to employing a terminal disclaimer, should the examiner maintain the rejection, once there is a finding of otherwise patentable subject matter.

**Claim Rejections – 35 USC § 112**

**PARAGRAPH 2, "Definiteness"**: Claims 16 and 33 stand rejected for alleged indefiniteness, apparently because the examiner objects to use of the phrase "such as." Applicants do not acquiesce to this rationale to rejection but do point out that the present claims are not subject to the examiner's objection. Accordingly, applicants request that the examiner withdraw the indefiniteness rejection in question.

**PARAGRAPH 1, "Enablement"**: The examiner rejects claims 18-43 and 52, contending that they do not have sufficient support in the specification. At page 4 of the Official Action, in particular, the examiner states that the application, while "enabling for peptides HpC, SYI, YPH or RPQ with six lysines (K (H) associated with cardiolipin (CDL) or diphosphoryl lipidae (DPL), or CHL, does not recently provide enablement for any positively charged protein and any negatively charged adjuvant...."

Applicants would direct the examiner's attention, however, to the fact that HpC is a protein antigen and not a "peptide," within the examiner's meaning. The association was shown to occur when the negative charge on the charged organic complex was increased by incorporation of DPL, an aspect unrelated to whether or not "six lysines" are present. (Indeed, the exemplified protein did not contain a six-lysine addition.)

At page 5 of the Official Action, the examiner alleges that, "while the specification teaches that mice had a CTL response in Examples 4, 6, 9, 14 and 17, there is no indication that the response was directed to the antigen specifically or to the particular ISCOMATRIX used to boost the response to the antigen." Applicants respectfully disagree with the examiner because, as a review of these examples reveals, the documented CTL

responses were generated specifically against the protein or peptide employed as a component of the immunizing formulation.

At page 25, lines 8-15, for instance, the specification indicates that splenocytes harvested from the immunized mice were re-stimulated with EL4 HHD cells that had been sensitized with ESO peptide and irradiated. These cells were cultured and then used as effectors in a standard $^{51}$CR-release assay against EL4 HHD cells sensitized as for re-stimulation. Similarly, each of Examples 6, 9, 14 and 17 detail cytotoxic T lymphocyte-response assays that were measured specifically against the immunizing protein or peptide. Thus, these examples provide every indication that the response generated by these mice was directed specifically to the immunizing antigen. In other words, these *in vitro* assays unequivocally demonstrate the generation of a CTL response to the immunizing peptide by the immunized mouse.

The examiner further alleges that the specification does not demonstrate that there is any predictability with how well proteins will associate, on the grounds that TYQ in Example 17 was treated to increase positivity by the addition of 6H and 6K, associated with a negatively charged adjuvant, and yet generated only very weak responses. Applicants submit, however, that the recombinant polytope of Example 17 contains six CTL epitopes in tandem. Responses to four of the six peptides were measured and reported. The results showed *strong* CTL responses to three epitopes and less strong responses to the fourth. It is respectfully submitted that:

(i) The construct used for immunization in this example was the recombinant polytope, not the individual epitopes.

(ii) The positivity of the polytope was increased by addition of the 6K and 6H. Such tags were not applied to the individual epitopes.

(iii) The success of the formulation was measured by determining CTL responses to certain of the component epitopes within the polytope.

(iv) A weak CTL response is nevertheless a positive response.

In summary, the formulation utilized in Example 17 did induce a *strong* CTL response to three of its component epitopes, and a weaker response to the fourth (TYQ). Thus, the overall CTL response generated to this polytope was a strong response, with relative

differences in response observed only when one investigated the extent of response to the individual epitopes comprising these polytopes.

In addition, applicants would direct the examiner's attention to Example 14, which describes studies with the equivalent synthetic polytope. Example 14 shows that good CTL responses were induced to peptide TYQ.

As to the citations made by the examiner, applicants note that Barr *et al.* discloses the use of conventional ISCOMS, into which antigens are introduced via hydrophobic regions or sequences. The very object of the present invention, however, is to obviate the need for introducing hydrophobic regions into antigens. Accordingly, Barr simply describes the difficulties that the present invention overcomes.

With regard to Offringa *et al.*, the examiner alleges that the use of peptides which have been emulsified in adjuvants were not always sufficient for inducing true anti-tumor immunity. But it is not clear how this reference, which relates to peptides emulsified in an adjuvant, is relevant to the subject matter of the present application, which teaches the induction of improved CTL responses where a charged organic carrier and a charged antigen are electrostatically associated.

At page 6 of the Official Action, the examiner alleges that undue experimentation would be required to make or use the claimed invention. Applicants respectfully disagree with the examiner's position on the ground that, in fact, only routine procedures would be required in this context.

In particular, the present application teaches means for bringing about association between a charged organic complex and charged antigen, means of increasing that association by modification of the organic complex, antigen or both, and means for testing that association. Accordingly, the application informs the skilled person of all technical means required to create the immunogenic complex. Further, the specification demonstrates that, when such an association is achieved, immunization routinely induces a CTL response.

**Claim Rejection – 35 USC § 102**

The examiner has rejected claims 1-5 for alleged anticipation by Seeber *et al.* and Al-Shakshir *et al.* Applicants would emphasize, however, that the teachings of Seeber *et al.* and Al-Shakshir *et al.* relate to aluminum salt adjuvants, *i.e.*, to ***inorganic*** carriers.

By contrast, the presently claimed invention concerns *organic* carriers, which are clearly different. The distinctions between organic and inorganic complexes are evident, for example, in light of the following points:

(i) An increasing of the association between aluminum salt gels and antigens involves modification to pH, ionic strength, ionic composition, gel size and nature of aluminum salt. With the exception of pH, however, none of these variables is relevant to the immunogenic complex as presently claimed, which comprises a charged organic carrier and a charged antigen.

(ii) The association of antigen with an aluminum salt leads to a *loss* of CTL induction. That is, the purpose of such an antigen/aluminum-salt association is to create an antigen depot, usually lasting around one week. Such an immunological outcome is quite different from what applicants believe is achieved with the immunogenic complexes of the present claims.

(iii) Although what the cited publications teach has been available for many decades, there is nothing in the prior art of record to suggest that the skilled artisan would have realized how or even whether these principles might apply to charged organic complexes.

Applicants submit, therefore, that the presently claimed invention indeed is patentable over any reasonable permutation of teachings drawn from Seeber *et al.* and Al-Shakshir *et al.* Accordingly, reconsideration and withdrawal of the pending anticipation rejection are warranted.

**Claim Rejections – 35 USC §§ 102 and 103**

Claims 1-8 and 12-14 are rejected over Nakanishi *et al.*, with the examiner asserting anticipation and obviousness in the alternative. On either ground, however, applicants would emphasize that Nakanishi *et al.* teach that *only positively charged liposomes* can induce CTL. (For example, see Figure 2, page 795 at column 1, paragraph 1, and the author's conclusions at page 796, column 2.) If anything, therefore, the cited Nakanishi document actually teaches away from the claimed invention.

Further, Nakanishi *et al.* used charged and neutral liposomes to investigate uptake by macrophages. There is no hint that this approach might pertain in binding charged antigens by electrostatic interactions. On this point, the examiner has stated that:

> Nakanishi *et al.* does not explicitly teach that antigens have a positive charge and are electrostatically associated with the negatively charged vesicle. However, the reference teaches that the negatively charged vesicles are composed of an amphipathic molecule, phosphatidylcholine. The negatively charged end of this molecule would be found in the interior of the vesicle, which associates directly with the protein antigen. Since some amino acids inherently have a positive charge, and these amino acids would be naturally attracted to the negative charge of the phosphatidylcholine, creating an electrostatic association between the antigen and the vesicle in some degree.

Applicants respectfully submit that the quoted commentary reflects a misunderstanding of liposomes structure and how liposomes can be loaded with antigen. In particular, liposomes consist of cholesterol and one or more phospholipids. In its simplest manifestation, as a small unilamellar vesicle (SUV), a liposome has a single bilayer membrane, with the charged head group exposed at the internal and external surface of the membrane. The phospholipid (PC) has both a positive and negative entity in its head group resulting in an overall neutral charge, *i.e.*, the liposome will be neutral at *both* its internal and external surface. Nakanishi *et al.* employed liposomes in the form of MLVs (multi-lamellar vesicles), which comprise a large number of concentric bilayer membranes. Loading of any liposome is generally achieved by vortexing or otherwise mixing a lipid film with an aqueous solution of antigen. Loading is very inefficient because the external aqueous volume after this process is vastly greater than the encapsulated aqueous volume.

Applicants submit that the prior art of record does not suggest this inefficiency could be overcome by selecting lipids for preparing liposomes that have a charge suitable for binding the chosen antigen to be loaded. Accordingly, the Nakanishi publication neither anticipates the claimed invention nor renders it obvious.

**Claim Rejections – 35 USC § 103**

The examiner has rejected claims 6-8 over Seeber *et al.* or Al-Shakshir *et al.* While admitting that neither reference teaches enhancement of the degree of charge for antigen or adjuvant, the examiner alleges that it would have been an obvious modification for one of ordinary skill in the art to perform.

Applicants traverse this rejection, again emphasizing that the examiner has cited art pertaining to *inorganic* charged carriers, which have a fixed charge, and has suggested that

one could add or subtract charged amino acid species in a protein to bring about association. The prior art does not presage this notion, in the context of the presently claimed invention. In the context of inorganic charged carriers, moreover, that posited addition/subtraction approach would have been deemed an undesirable step to take, on the ground that it could lead to critical changes within specific CTL epitopes, such that they no longer would be recognized by MHC I.

The examiner has rejected claims 9-11 and 15-17 over Nakamishi *et al.*, as applied to claims 1-8, 12-14, read in view of Barr *et al.* The examiner alleges that Barr *et al* review different ISCOMs, and name saponin, lipid A, and phospholipids, which are taught by Nakamishi *et al.*, as obvious variants to one another.

To illuminate this point, applicants would clarify the role of saponins and phospholipids within ISCOMs, and what outcome could be desired if MPL (the monophospholyl derivative of lipid A) were included. Interaction between saponin and cholesterol causes formation of a ring structure, about 10 nm in diameter, that, in the presence of lipids (preferably phospholipids) and under fairly broad stoichiometric constraints, self-assemble into 40 nm, open cage-like structures. These complexes have two important properties. They contain Quillaia saponins, which are powerful immunomodulators, and they comprise hydrophobic regions, where hydrophobic antigens can be bound. *Thus, phospholipids are not variants of saponins.* Rather, phospholipids are normal components of cell membranes and lack immunological activity. Saponins disrupt cell membranes, and one subset, Quillaia saponins, have strong immunomodulating activities.

Suggestions have been made that MPL could be incorporated into ISCOMS to increase the Th1-modulating activity of the complex. To applicants' knowledge, however, no one has suggested that MPL, DPL, or another lipid could be incorporated into ISCOMs to increase their negative surface charge, thereby to aid in binding antigens by electrostatic interaction. Accordingly, the prior art taken as a whole does not render the claims in question obvious, within the meaning of Section 103.

Still further, the examiner has rejected claims 18-43 and 52 over Seeber *et al.* or Al-Shakshir *et al.* and Nakanashi *et al.*, as applied to claims 1-17, in view of Barr *et al.* Applicants submit, however, that the examiner's analysis and his combining of the documents in question are informed by improper hindsight. Essentially for the reasons

developed above, including the fact that many of cited documents teach away from the presently claimed invention and/or relate to inorganic charged complexes. Despite the teachings of Seeber *et al.* and others in relation to formulating aluminum salt-adjuvant vaccines, moreover, there is long-felt need, for means to induce a CTL response, that has not been met heretofore but that the present invention satisfies. Accordingly, the pending obviousness rejections must be seen as ill-founded and warranting withdrawal.

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Based on the foregoing, applicants submit that the present claims are in allowable condition, and favorable reconsideration of the application therefore is requested. The examiner is invited to contact the undersigned, should there be any other issues that may require consideration.

Respectfully submitted,

November 23, 2001
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## Versions with Markings to Show Changes Made

1 (Amended). An immunogenic complex comprising a charged organic [carrier] complex and a charged antigen, which organic [carrier] complex and antigen are electrostatically associated.

2 (Amended). The immunogenic complex according to claim 1 wherein said [carrier] charged organic complex is negatively charged and said antigen is positively charged.

4 (Amended). The immunogenic complex according to claim 2 wherein said [carrier] charged organic complex is an adjuvant or derivative or equivalent thereof.

5 (Amended). The immunogenic complex according to claim 2 wherein said antigen is a protein or derivative or equivalent thereof and said [carrier] charged organic complex is an adjuvant or derivative or equivalent thereof.

6 (Amended). The immunogenic complex according to claim 5 wherein said [negatively charged] adjuvant is a naturally negatively charged adjuvant which has been modified to increase the degree of its negative charge.

7 (Amended). The immunogenic complex according to claim 5 wherein said [positively charged] protein is a naturally positively charged protein which has been modified to increase the degree of its positive charge.

8 (Amended). The immunogenic complex according to claim 5 wherein said [negativley charged] adjuvant is a naturally negatively charged adjuvant which has been modified to increase the degree of its negative charge and said [positively charged] protein is a naturally positively charged protein which has been modified to increase the degree of its positive charge.

16 (Amended). The immunogenic complex according to claim 15 wherein the lipid A is selected from the group consisting of diphosphoryl lipid A [such as OM174] and monophosphoryl lipid A.

18 (Amended). A vaccine composition comprising as the active component a charged organic [carrier] complex and a charged antigen, which [carrier] charged organic complex and antigen are electrostatically associated, together with one or more pharmaceutically acceptable carriers and/or diluents.

19 (Amended). The vaccine composition according to claim 18 wherein said [carrier] charged organic complex is negatively charged and said antigen is positively charged.

21 (Amended). The vaccine composition according to claim 19 wherein said [carrier] charged organic complex is an adjuvant or derivative or equivalent thereof.

22 (Amended). The vaccine composition according to claim 19 wherein said antigen is a protein or derivative or equivalent thereof and said [carrier] charged organic complex is an adjuvant or derivative or equivalent thereof.

23 (Amended). The vaccine composition according to claim 22 wherein said [negatively charged] adjuvant is a naturally negatively charged adjuvant which has been modified to increase the degree of its negative charge.

24 (Amended). The vaccine composition according to claim 22 wherein said [positively charged] protein is a naturally positively charged protein which has been modified to increase the degree of its positive charge.

25 (Amended). The vaccine composition according to claim 22 wherein said [negatively charged] adjuvant is a naturally negatively charged adjuvant which has been modified to increase the degree of its negative charge and said [positively charged] protein

is a naturally positively charged protein which has been modified to increase the degree of its positive charge.

33 (Amended). The vaccine composition according to claim 32 wherein the lipid A is selected from the group consisting of diphosphoryl lipid A [such as OM174] and monophosphoryl lipid A.